13384-002001

STATUTORY DECLARATION

I, James Alun Wynne Morgan, Warwick HRI, University of Warwick, Wellesbourne, Warwick CV35 9EF, declare and state that:

1. I am a project leader at Warwick HRI (formally Horticulture Research International), which position I have held since 1992.
2. I hold the degrees of Bachelor of Science (Hons, 1st Class) in Applied Biology conferred on me from the Liverpool Polytechnic (now John Moores University); and the degree Doctor of Philosophy conferred on me from Liverpool University.
3. From 1989 to 1992 I was a research fellow at the Freshwater Biological Association, Ambleside, Cumbria.
4. As well as my administrative and project leader roles, I am active in supervision of post-graduate MSc and PhD research projects.
5. I am the author or co-author of many original publications including research papers and book chapters, and I have presented papers at many international meetings.
6. In the course of my professional activities I attend national and international conferences relevant to my field of research and interest. At such conferences I meet with colleagues from the UK and abroad. This affords me the opportunity to discuss and familiarise myself with the current state of the art and new developments, both conceptual and technological.
7. I have reviewed prior to making this declaration a copy of the Patent Application (US 09/889,874) and letters and emails relating to percent similarity.
8. I believe that those skilled in the art such as myself would clearly understand that it is suitable to use DNA hybridization techniques to isolate genes that belong to a similar general group using DNA hybridization. The techniques that I and many others have used, are well documented and most experiments follow standard methods set out in classic textbooks such as that of Sambrook, Fritsch and Maniatis, Molecular cloning a laboratory manual (second edition), 1989 Cold Spring Harbor Laboratory Press, and earlier versions of this manual. In this method DNA from bacteria to be tested is fixed to a nylon or nitrocellulose filter and a probe made from the target gene is hybridized to the target DNA. To enable conditions to be established various information can be considered but the following material; is particularly useful. The equation: $Tm = 81.5°C - 16.6(\log10[Na+]) + 0.41 (\%G+C) - 0.63 (\%\text{ formamide}) - (600/l)$ can be used to calculate the Tm of the probe provided Na+ concentration is in the range of 0.01M to 0.4M; G+C content is between 30% to 70%, and l is the length of the probe helps establish the conditions required. The equation can also be reversed to help determine the washing conditions to be used. The Tm of a double stranded DNA hybrid decreases by 1-1.5°C with every 1% decrease in homology (Bonnar *et al.*, 1973; reduction in the rate of DNA re-association by sequence divergence. J. Mol. Biol. 81:123). In this way rough experimental conditions can be established where segments of DNA with very similar or very different DNA sequence can be isolated.